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Heavy metals bioaccumulation analysis with macroalgae growing in Ganga water in Prayagraj, Uttar Pradesh, India

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Abstract

For a sustainable ecosystem and the benefit of mankind, knowledge of environmental pollution with a health impact is crucial. Industrial waste and sewage sources contaminate the Ganga River's water. Freshwater algae have a well-known ability to bioaccumulate heavy metals. We investigated the ability of the green algae *Hydrodictyon reticulatum* and *Rhizoclonium* sp. to biologically accumulate heavy metals in an aqueous environment as a response to the increasing environmental contamination associated with heavy metals. We analysed the heavy metals Cd, Cr, Pb, and Fe. We collected algal biomass and Ganga water samples from Prayagraj, Uttar Pradesh, in March 2022 to study heavy metals. Heavy metals were analyzed using a spectrophotometric technique and an atomic absorption spectrometer (Perkin Elmer A-700). Green algae can highly accumulate heavy metals. We examined heavy metals in algal and Ganga water samples. We found the concentrations of heavy metals in Ganga water in the following order: Cd > Cr > Pb > Fe. In *Hydrodictyon reticulatum*, the bioaccumulation factor was 169.16 for Fe, 27.5 for Pb, 22.14 for Cr, and 2.58 for Cd. In *Rhizoclonium* sp., it was 352.5 for Fe, 34.74 for Pb, 27.63 for Cr, and 3.13 for Cd.

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Introduction

The issue of groundwater pollution has grown to be of great concern to the public, organizations, and governments (Wanjun, 2022). Public health and national sustainable development are becoming increasingly dependent on water quality safety, and people are becoming more conscious of this (Shengfeng *et al.*, 2019; Wang *et al.*, 2017; Wang *et al.*, 2017; Siwen, 2022). Thus, it is crucial to conduct pertinent research to build an ecological ecosystem and safeguard local groundwater resources (Qifa *et al.*, 2022).

Chemical contamination of water bodies is one of the most serious problems facing aquatic ecosystems worldwide. Chemical contamination of water bodies is one of the most important problems affecting aquatic ecosystems globally. A few heavy metals have gained significant importance in toxicological and environmental chemistry research. These heavy metals include Cd, Cr, and Pb, which are usually poisonous even at very low levels, as well as potentially hazardous metals (Tamayo *et al.*, 2014). Rocks naturally contain relatively small amounts of metals. They play significant roles in our daily lives and possess desirable attributes. Heavy metals are a widely used term for metals and metalloids with an atomic density greater than 4 g/cm3. We occasionally use the term "toxic heavy metals" to emphasize how these substances affect the environment, particularly the biota (the biological approach). Sometimes, to emphasize how these elements impact the environment and specifically the biota (the biological approach), the term "toxic heavy metals" is used. Heavy metals can be categorized as hazardous because of their harmful effects on living things. Due to their important involvement in the metabolic processes that occur in living cells, some heavy metals, such as copper, nickel, and zinc, are known as microelements or trace elements and are essential to life at very low concentrations (Gadd, 1993). But at high concentrations, most prokaryotic and eukaryotic species are toxic to these metal ions. It is not required to use other heavy metals like cadmium, lead, or mercury because studies have shown that even at very low concentrations, these metals seriously damage living things. The majority of research on metals (Sunda, 1975; Anderson *et al.*, 1978) suggests that the free ion is the species most harmful to aquatic life. A variety of human activities, including intensive farming, metallurgy, energy production, the production of microelectronics, and the disposal of sewage sludge, cause heavy metal pollution. Heavy metal pollution remains stable and persistent due to its inability to break down or degrade. Therefore, the toxicity of heavy metals severely harms the environment and human health, necessitating a constant search for practical and cost-effective methods to detoxify metal-contaminated areas (Kaplan, 2013).

Contemporary technology uses biological methods, which are more sustainable and environmentally friendly, to remove heavy metals to combat this issue (Kumar *et al.*, 2022). Algae are abundant, reasonably priced, highly efficient at removing metals, safe for the environment, and capable of producing products with additional economic value, making them a viable approach for heavy metal recovery. Macroalgae and microalgae are the two categories of algae that are commonly recognized. Redha (Ali Redha, 2020) primarily divides microalgae into four categories: diatoms, green, golden, and blue-green algae. Macroalgae are classified into three types: brown, red, and green.

The ability of macroalgae to absorb heavy metals can lead to concentrations hundreds of times higher than those found in river water. Tamayo (Tamayo *et al.*, 2014) asserts that suspended particulate matter (SPM), a blend of organic and inorganic complexes, shapes the morphologies of algae, exclusively storing free metal ions. Algae are also sedentary; they are the right size, easily detectable and collected, extensively dispersed, and capable of sufficiently accumulating metals. These attributes make algae ideal bioindicators (Tamayo *et al.*, 2014). Algae exhibit detoxifying, bioaccumulating, and biosorption processes that remove most harmful substances. Heavy metals accumulate within cells through a process known as bioaccumulation. Living cells carry out the accumulation process, which depends on metabolism and requires energy. Thus, bioaccumulation is an active intracellular process that algae use as a self-defence mechanism (Chugh *et al.*, 2022). Compartmentalization, in contrast to bioaccumulation, is the term for the collection of ions, specifically in the cell organelles (vacuoles or thylakoids) (Zohoorian *et al.*, 2020).

Materials and methods

Study area

The city of Prayagraj is situated in the southern region of Uttar Pradesh. The geographical coordinates of this study area are 25.505° N latitude and 81.868° E longitude, about northwest of Prayagraj City. Out of all the rivers, the Ganga is the most sacred. Millions of Indians who live along the riverbank and rely on it for their everyday necessities moreover regard it as a lifeline. Hinduism worships it, personifying it as the goddess Ganga. Millions of pilgrims to the sacred Ganges River at Haridwar, Prayagraj, and Varanasi, three mythologically significant Hindu cities, hoping to cleanse their sins and attain redemption (Pandey *et al.*, 2014). There have been significant pollution issues along the Ganga between Kanpur and Prayagraj (formerly Allahabad). Ganga pollution is mostly caused by industrial discharge, urban runoff, agricultural runoff, tanneries, and chemical plants in Kanpur city. The Ganga transports industrial pollution downstream to Prayagraj (Fig. 1 A), where it is further contaminated by industrial and home waste, ritual baths, and numerous spiritual activities (Aggarwal *et al.*, 2022).

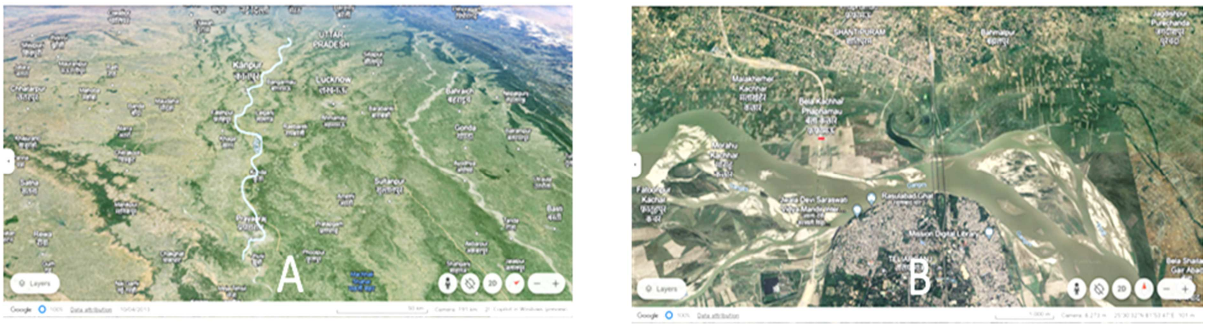

Fig. 1. A. Google Earth image showing a stretch of River Ganga from Kanpur to Prayagraj; B. Google Earth image showing a stretch of River Ganga in Prayagraj city

Fig. 2A-D. Collection of algal samples from Ganga water

Fresh and wet algal biomass was collected from Ganga River Phaphamau Prayagraj, UP (25.505° N, 81.868° E) in March 2022 (Fig. 1B & 2). Systematic authentication of the algal samples was carried out by microscopic studies (Blisco India an ISO 900: 2015) at Botany Department CMP Degree College Prayagraj and the status assigned to the samples are *Rhizoclonium* sp. i.e., sample-1, *Hydrodictyon reticulatum* i.e. sample-2. The wet algal biomass was manually washed several times with water to remove its impurities like sand, leaves, wood, feathers, etc. After washing the biomass was dried in natural sunlight for 2 days (moisture content was reduced to 20% from 80%) followed by drying in an oven at 45 °C for 24 hours, after which the milling of the sample was done and the fraction with 212–500 µm particle size was packed in plastic bags.

Pretreatment and AAS analysis of heavy metals in algal samples

The dried samples were digested with HNO_3:$HClO_4$ (3:1, v/v) at 80 °C and then diluted with Milli Q water (Dwivedi *et al.*, 2010). The digested samples were analyzed for different metals viz. Cd, Cr, Pb & Fe by measuring the absorbance of samples on an atomic absorption spectrophotometer (Perkin Elmer A700). The total metal concentrations in algal samples and Ganga water were detected after filtration (using Whatman filter paper no. 41, 20-25µm) for removal of debris and other organisms. The digestion and analysis of the samples were done as described above for algal samples. The following formulae evaluated metals enrichment factor in algal species:

$$Ef = \frac{[*Cm]algae}{[*Cm]water}$$

Ef = Enrichment factor

$*C_m$ = concentration of the metal in algae and water

Statistical analysis

In each case, three replicas of each determination were made. Analysis of variance (ANOVA<0.05) and Duncan's multiple range test (DMRT<0.05) were used to assess for significant variations in metal accumulation between the two algae species to confirm the validity of the findings.

Observations

Systematic authentications of the algal samples were carried out at the Botany Department CMP Degree College Prayagraj. The status assigned to the samples is *Rhizoclonium* sp. and *Hydrodictyon reticulatum* (Fig. 3 A, B).

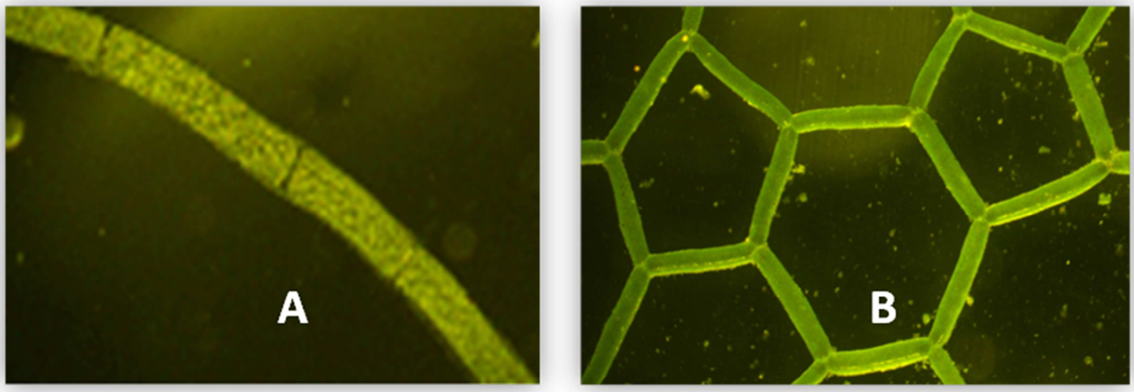

Fig. 3. A. *Rhizoclonium* sp. B. *Hydrodictyon reticulatum*

Results and Discussion

The bioaccumulation potential of green macroalgae for heavy metals and the water quality of River Ganga have been presented in this work. Physicochemical properties of water have been done by water analyzer (HANNA HI98196-web), sulfate and nitrate concentrations have been analyzed by turbidimetric method, and phosphate concentration has been analyzed by spectrophotometric method (Table 1).

Cadmium

Cadmium metal is used in the steel and plastic industries. Cadmium compounds are extensively utilized in batteries. Cadmium is released into the environment through wastewater, while fertilizer contamination and local air pollution contribute to diffuse pollution. Cadmium is most commonly consumed through food. Smoking is an important additional cause of cadmium exposure. WHO has proposed a total cadmium concentration of 0.003 mg/l in drinking water (WHO Guidelines, 2017).

Table 1. Analysis of Ganga water sample using water analyzer

S.N.	Parameters	Value
1.	mVpH	-88.5
2.	pH	8.57
3.	mVORP	207.4
4.	% DO Saturation	1.8
5.	DO Conc.	0.13
6.	Conductivity	352
7.	Absolute Cond.	391
8.	Resistivity	0.0028
9.	TDS	176
10.	Salinity	0.17
11.	Sea water	0
12.	Temp.	30.78
13.	Atm. Pressure	14.486
14.	PO_4^{3-}	0.76 mg/l
15.	NO_3^-	7.67 mg/l
16.	SO_4^{2-}	14.88 mg/l

Table 2. Maximum permissible limits for heavy metals concentration in water (WHO Guidelines, 2017; Council of the EU, 1998; BIS, 2012)

Heavy metals	WHO (mg/L) *	EU (mg/L) **	BIS (mg/L) ***
Cadmium	.003	.005	.003
Chromium	.05	.05	.05
Lead	.01	.01	.01
Iron	-	-	.3

*World Health Organization (2017)

**Council of the European Union (1998)

***Bureau of Indian Standards (2012)

Chromium

Chromium is found in abundance throughout the Earth's crust. Its valence ranges from +2 to +6. Food is provided to be the most common source of intake. There are not enough toxicity studies to support a NOAEL (no observable adverse effect level). The guideline value for hexavalent chromium was first proposed in 1958 due to health concerns, but it was later revised to a guideline for total chromium due to challenges in analyzing the hexavalent form alone. WHO recommends a total chromium content of 0.05 mg/l in drinking water. Because of uncertainties in the toxicological database, this result has been categorized as provisional (WHO Guidelines, 2017) (Table 2).

Lead

Lead is primarily utilized in the manufacture of lead-acid batteries, solders, and alloys. Tetraethyl and tetramethyl lead, two organolead compounds, were also widely employed as antiknock and lubricating additives in gasoline, though their use in many nations has been eliminated. Lead is rarely present in tap water since it dissolves naturally. Free chlorine residuals in drinking water tend to accumulate more insoluble lead-containing sediments, whereas chloramine residuals may produce more soluble sediments in lead pipes. It is highly difficult to attain a lower concentration through central conditioning, such as phosphate dosing; therefore, the guideline value of 0.01 mg/l is retained but is marked as provisional based on treatment performance and analytical achievability (WHO Guidelines, 2017).

Iron

Iron is one of the most plentiful metals in the earth's crust. It is present in natural freshwater at concentrations ranging from 0.5 to 50 mg/l. Iron may also be found in drinking water due to the usage of iron coagulants or corrosion of steel and cast-iron pipelines during water distribution. Iron is a vital nutrient in human nutrition, especially in the iron (II) oxidation state. The minimal daily requirement for iron varies depending on age, gender, physiological status, and iron bioavailability, ranging from roughly 10 to 50 mg/day. WHO has not suggested a recommended value for iron in drinking water (WHO Guidelines, 2017).

The surface water collected from Ganga River, Prayagraj was significantly contaminated as it was highly alkaline (pH 8.57) and had high values of total dissolved solids (TDS; 176mgL−1), %DO saturation (%DO; 1.8), dissolved oxygen (DO; 0.13mgL−1), phosphate (PO43-; 0.76 mgL−1), nitrate (NO3-; 7.67 mgL−1), sulphate (SO42-; 14.88 mgL−1), salinity 0.17, temperature 30.78°C (Table 1).

Table 3. Metal concentration in bloom (high biomass) producing green algae collected from heavy metal contaminated site

S. No.	Parameters	Ganga water	*Hydrodictyon reticulatum*	*Rhizoclonium* sp.
1.	Cd	33.5	86.63±0.48[b]	104.56±0.43[b]
2.	Cr	1.77	39.20±0.49[c]	61.50±0.50[c]
3.	Pb	BDL	27.50±0.50[d]	27.63±0.60[d]
4.	Fe	BDL	169.16±0.82 [a]	352.50±0.80[a]

The data was statistically tabulated and tested at 0.05 level of significance (ANOVA and Duncan's multiple range test) where n= 3

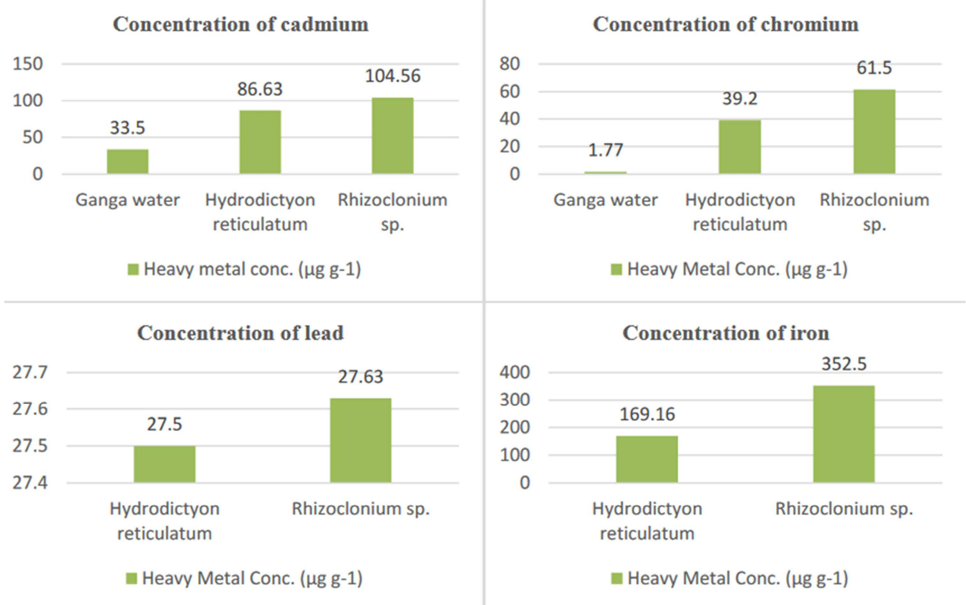

Fig. 4A-D. Concentration of heavy metals in Ganga water and green macroalgae

Heavy metals were examined in algal and Ganga water samples. The concentrations of heavy metals in Ganga water were found in the following order: Cd > Cr > Pb > Fe (Table 3). The concentration of lead and iron is in very little quantity as it was below the detection level in Ganga water. The concentration of heavy metals in *Hydrodictyon reticulatum* was found in the following order: Fe> Cd> Cr> Pb and the values are 169.16> 86.63> 39.20> 27.50 respectively (Fig. 4 A-D). The concentration of heavy metals in *Rhizoclonium* sp. follows the same order of accumulation as in *Hydrodictyon reticulatum,* i.e., Fe> Cd> Cr> Pb, and the values are 352.50> 104.56> 61.50> 27.63 respectively (Fig. 4 A-D). The bioaccumulation factor recorded in *Rhizoclonium* sp. is 352.5, 34.74, 3.13, and 27.63 for Fe, Cr, Cd, and Pb respectively and in *Hydrodictyon reticulatum* the bioaccumulation factor was, 169.16, 27.5, 22.14 and 2.58 for Fe, Pb, Cr, and Cd respectively (Table 4; Fig. 5).

Table 4. Bioaccumulation factor of various heavy metals in green algae

Heavy metals ($\mu g\ g^{-1}$)	*Hydrodictyon reticulatum*	*Rhizoclonium* sp.
Cd	2.58	3.13
Cr	22.14	34.74
Pb	27.50	27.63
Fe	169.16	352.50

From the above experimental results, we can now conclude that both the green macroalgae are very efficient sources of heavy metals bioaccumulation in aquatic ecosystems but *Rhizoclonium* sp. is showing better accumulation capacity than previous alga. However, prolonged exposure of the algae to surface water is primarily responsible for a higher accumulation of macroalgae compared to surface water in natural conditions. Algae are the hyperaccumulators of heavy metals and the source of O_2 in the aquatic atmosphere.

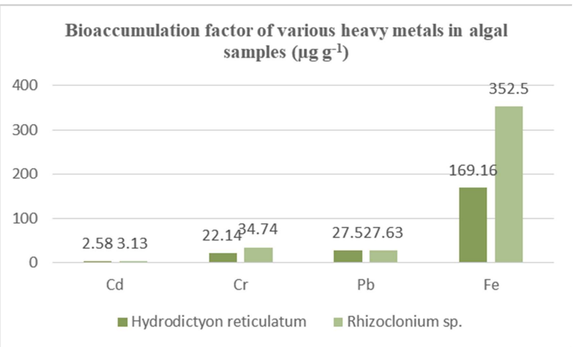

Fig. 5. Bioaccumulation factor of various heavy metals in algal samples

These species produce a large amount of biomass, which can be further used for remediation and to produce innovative products like biodiesel, biomethane, organic fertilisers, feedstock, nanoparticles, and pharmaceutical products. Strains vary in their physical and metabolic properties, leading to variations in heavy metal remediation methods. Two mechanisms, biosorption and bioaccumulation, play a major role in the abating of heavy metals. The detoxification mechanism converts toxic heavy metals into non-toxic ones after bioaccumulation, and the efflux mechanism transports them out of the cell. Thus, processes like adsorption, accumulation, complexation, compartmentalisation, chelation, and reduction play an important role in the remediation of heavy metals. To fully explore the heavy metal resistance mechanism of algae and enhance the rate of remediation and by-product production, further molecular studies are necessary.

Conclusion

The Ganga is becoming more and more contaminated every day as a result of human activity and untreated industrial runoff. Heavy metals remain in the environment for a prolonged period due to their inability to break down, severely contaminating aquatic environments. The current study investigates the accumulation of heavy metals by green algae, a cost-effective method for removing pollutants from aquatic environments. The quantity of heavy metals found in the city's Ganga water helps the government determine how contaminated the water is and how to remove heavy metals from it using algae. It also assists the government in cleaning up the Ganga water at a very reasonable cost. Based on this research, it is evident that employing algae to remove heavy metal pollution will become a viable and highly efficient method in the future.

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